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Short communication

Antioxidant activity of high sulfate content derivative of ulvan in hyperlipidemic rats

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ABSTRACT

High sulfate content derivative of polysaccharide (HU) from *Ulva pertusa* (Chlorophyta) showed strong antioxidant activity *in vitro*. In the present study, *in vivo* antioxidant activity were tested in liver of hyperlipidemic rats including malondialdehyde (MDA), superoxide dismutase (SOD), glutathione peroxidase (GSH-Px) and catalase (CAT). The HU groups at the doses of 125 mg/kg and 250 mg/kg showed stronger activity on SOD than hyperlipidemimia group ($P < 0.01$). The HU groups at the doses of 125 mg/kg and 500 mg/kg could increase the activities of GSH-Px obviously ($P < 0.01$) as compared with hyperlipidemic rats. It was likely that the sulfate content had significant effect on the antioxidant activity *in vivo*. On the other hand, it may be concluded that, probably due to its antioxidant effects, HU is effective in the protection of liver tissue from the damage of cholesterol-rich diet rats and that the HU may be of use as an antihyperlipidemia agent.

1. Introduction

Oxidative stress and reactive oxygen species (ROS) have been associated with a variety of chronic health problems such as cardiovascular disease, certain cancers, malaria, rheumatoid arthritis, diabetes, Alzheimer's disease, Parkinson's disease, other neurological disorders and also aging process [1]. Chen et al. [2] reported that long-term high-fat diet treatment could increase the production of reactive oxygen, develop lipid peroxidation, oxidative injury. Yamatoya et al. [3] found that some oligosaccharides can reduce effectively oxidative stress in high-fat diet-treated rats or in streptozotocin-induced diabetic rats. Polysaccharides from Curcuma kwangsiensis could protect the experimental animals against oxidative injury induced by high-fat diet treatment [4].

Antioxidants, which scavenge free radicals, are known to play import roles in preventing ROS-induced diseases [5]. In recent years, sulfated polysaccharides from marine algae have been reported to have antioxidant activates. Zhang et al. [6] found that the sulfated polysaccharide fraction F2 from *Porphyra haitanesis* can be used in compensating the decline in TAOC and the activities of antioxidant enzymes and thereby reduces the risks of lipid peroxidation. Sulfated polysaccharide from *Enteromorpha linza* was also demonstrated to have free radical scavenging activities [7]. On the other hand, the commonly used synthetic antioxidants such as BHA (buthylated hydroxyanisole) and BHT (buthylated hydroxytoluene) are restricted by legislative rules because they are suspected to have some toxic effects and as possible carcinogens as well as general tendencies in consumer preferences towards naturalness of foods [8]. Compared with the synthetic antioxidants, natural polysaccharide may be safer as antioxidant food additives.

Ulva pertusa, green alga, is distributed in China in the intertidal zone of the Yellow Sea and Bohai Sea. Moreover, it is an important herbal drug, prescribed in the Supplement to Compendium of Materia Medica [9], which has been used for treatment of hydropic. The polysaccharide extracted from *U. pertusa* is a group of sulfated heteropolysaccharides and the main disaccharide units are [β-D-GlcpA-(1 → 4)-α-L-Rhap3s] and [α-L-Idop A-(1 → 4)-α-L-Rhap 3s] [10]. During the last years, polysaccharide extracted form *U. pertusa* has been reported to have antihyperlipidemic, antitumor, antiviral and antioxidant activities [10–14]. For simplicity, the sulfated polysaccharide is referred to as ulvan (U) in our paper. In our previous study, ulvan and high sulfate content polysaccharide derivative (HU) both have antioxidant and antihyperlipidemic activities, furthermore, HU showed stronger activity than ulvan [11,12]. The antioxidant activity of HU suggested it may be helpful in retarding the antihyperlipidemia. In this study, we investigated the *in vivo* antioxidant activity of U and HU in cholesterol-rich diet treated rats.

Abbreviations: CAT, catalase; GSH-Px, glutathione peroxidase; HDL-C, high density lipoprotein cholesterol; HU, high sulfate content ulvan; LDL-C, low density lipoprotein cholesterol; MDA, malondialdehyde; SOD, superoxide dismutase; TC, total cholesterol; TG, triglyceride; U, ulvan.

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Table 1
Effects of U, HU and colestyramine on serum lipid profiles in rats supplemented with a cholesterol-rich diet.

Group	Dose (mg/kg)	TC (mmol/L)	TG (mmol/L)	LDL-C (mmol/L)	HDL-C (mmol/L)
Normal control	–	1.78 ± 0.23	0.90 ± 0.14	0.49 ± 0.13	1.26 ± 0.14
Hyperlipidemia	–	2.75 ± 0.47$^{\Delta\Delta}$	1.29 ± 0.11$^{\Delta\Delta}$	0.70 ± 0.21$^{\Delta}$	0.88 ± 0.29$^{\Delta\Delta}$
U	250	2.08 ± 0.31	0.86 ± 0.19*	0.47 ± 0.19*	1.09 ± 0.23*
HU (low dose)	125	1.86 ± 0.49*	0.73 ± 0.09$^{**,\#}$	0.52 ± 0.11*	1.09 ± 0.24*
HU (middle dose)	250	1.80 ± 0.29**	0.83 ± 0.18*	0.39 ± 0.16**	1.03 ± 0.45
HU (high dose)	500	1.83 ± 0.25**	0.80 ± 0.14*	0.42 ± 0.13*	1.10 ± 0.34*
Positive control	500	2.06 ± 0.53*	1.06 ± 0.23	0.54 ± 0.18*	1.13 ± 0.13*

$^{\Delta}$ $P<0.05$: Compared with normal group.
$^{\Delta\Delta}$ $P<0.01$: Compared with normal group.
* $P<0.05$: Compared with hyperlipidemia control group.
** $P<0.01$: Compared with hyperlipidemia control group.
$^{\#}$ $P<0.05$: Compared with ulvan group.

2. Materials and methods

2.1. Materials and chemicals

U. pertusa was collected on the coast of Qingdao, China, in October, 2012. The algae were washed, air dried and kept in plastic bags at room temperature in a dark place before use. Polysaccharide extracted from *U. pertusa* (U) and high sulfate content ulvan derivative (HU) were prepared as described previously [12]. The sulfate contents of U and HU were 19.5% and 32.8%, respectively.

The assay kits for serum cholesterol (TC), triacylglycerols (TG), high-density lipoprotein cholesterol (HDL-C) and low-density lipoprotein cholesterol (LDL-C) were purchased from Shanghai Rongsheng (China). Assay kits for protein, malondialdehyde (MDA), superoxide dismutase (SOD), glutathione peroxidase (GSH-Px) and catalase (CAT) were obtained from Nanjing Jiancheng Bioengineering Institute (China).

2.2. Animals and experimental design

Eighty-four Wistar rats (male/female, 180–220 g) were provided by the Animal Lab Center of Shandong University (number of animal license SCXK (Lu) 20090001) (China). The animals were maintained in stainless steel cages at room temperature (25 ± 2 °C) and 12 h light cycle, and were allowed free access to standard laboratory pellet diet and water during the experiments. On the other hand, the current study protocol was approved by Ethics Committee of Weifang Medical University for animal studies.

After all the experiment rats were fed the standard laboratory pellet diet for 3 days, they were randomly divided into 7 groups ($n=12$) and began to feed a cholesterol-rich diet except for the normal control group. Group 1 was normal control while group 2 served as hyperlipidemia control and group 3 had animals treated with U (250 mg/kg body weight). Groups 4–6 received HU in doses of 125 mg/kg, 250 mg/kg and 500 mg/kg whereas group 7 had the standard drug (colestyramine, 500 mg/kg) treated animals that served as positive control. At the same time, group 3–7 were given different dose of U, HU and colestyramine by oral administration for 28 days. At the end of the experimental period (28 days), the rats were withheld food for at least 12 h, weighed and blood samples were collected from the eyeballs to measure TC, TG, HDL-C and LDL-C levels by the described kits methods.

The composition of cholesterol-rich diet was 2.0% cholesterol, 8.0% lard, 0.3% sodium cholic acid and 89.7% commercial chow. Animals had free access to water and food *ad libitum*.

The activities of MDA, SOD, GSH-Px and CAT were assayed according to the commercial kit manufacturer's instructions.

2.3. Statistical analysis

The data were presented as means ± S.D. and evaluated by one-way ANOVA followed by the Student's *t*-test to detect inter-group differences. Differences were considered to be statistically significant if $P<0.05$.

3. Results and discussion

3.1. Antihyperlipidemic activity in rats

As shown in Table 1, compared with hyperlipidemia group, the results indicated that HU-fed group (125 mg/kg) had optimal effect on TG ($P<0.01$), but a lesser impact on TC, LDL-C and HDL-C. However, doses of 250 mg/kg and 500 mg/kg had significant effects on TC ($P<0.01$). More importantly, the antihyperlipidemic activity of dose at 125 mg/kg was the strongest, compared with normal group, TG concentrations were significantly decreased by 18.89% ($P<0.05$). The results proved that the antihyperlipidemic activity was not concentration dependent for HU-fed rats.

3.2. The spleen and fat indices

Table 2 showed the effects of U, HU and colestyramine on spleen and fat indices in hyperlipidemic rats. Compared with normal or hyperlipidemia groups, there were no significant changes on spleen index, while the fat index of hyperlipidemia group markedly increased ($P<0.01$). Compared with hyperlipidemia group, treating hyperlipidemia rats with U produced a significant decrease of fat index ($P<0.05$).

3.3. Antioxidant activities in vivo of U, HU and colestyramine

The MDA production, a main index of lipid peroxidation, was decreased of all samples compared with hyperlipidemia group (Table 3). However, the MDA levels of all groups were increased compared with normal group. HU treatment at high

Table 2
Effects of U, HU and colestyramine on the spleen and fat indices in rats.

Group	Dose (mg/kg)	Spleen index	Fat index
1. Normal control	–	0.220 ± 0.080	0.594 ± 0.084
2. Hyperlipidemia	–	0.216 ± 0.092	1.074 ± 0.031$^{\Delta\Delta}$
3. U	250	0.235 ± 0.024	0.768 ± 0.042*
4. HU (low dose)	125	0.243 ± 0.010	0.835 ± 0.097$^{\Delta}$
5. HU (middle dose)	250	0.206 ± 0.019	0.772 ± 0.185
6. HU (high dose)	500	0.248 ± 0.010	0.808 ± 0.191
7. Positive control	500	0.218 ± 0.011	0.887 ± 0.105$^{\Delta}$

$^{\Delta}$ $P<0.05$: Compared with normal group.
$^{\Delta\Delta}$ $P<0.01$: Compared with normal group.
* $P<0.05$: Compared with hyperlipidemia control group.